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Study of the Antibacterial Activity of Total Extract and Petroleum Ether, Chloroform, Ethyl Acetate and Aqueous Fractions of Aerial Parts of *Heliotropium bacciferum* against *Staphylococcus aureus*, *Bacillus cereus*, *Pseudomonas aeruginosa*, *E.coli, Salmonella enteritidis*

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Heliotropium bacciferum is One of the plants belonging to the family Boraginaceae , which is Restricted distribution in the south of Iran. It is used for Hypotension, fever, stomach ulcers in traditional medicine. In this study, the antibacterial effects of extracts and fractions of chloroform, ethyl acetate and aqueous, aerial parts of Heliotropium bacciferum Forssk was evaluated against five bacterial strains. The methanol extract were prepared using the percolation method. Fractions of chloroform, Petroleum ether, ethyl acetate, methanol and aqueous respectively by Liquid - Liquid fractionation of the total extract were prepared. The antibacterial activity against two Gram positive bacteria, three Gram negative bacterial using Minimum inhibitory concentration in microplate and well plate method. Results showed that H. bacciferum extracts exhibited a significant activity against strains Staphylococcus aureus, Bacillus cereus,Pseudomonas aeruginosa, E.coli, Salmonella enteritidis. MIC and well plate is between 7.6-125 μg/ml. The results of this study indicate that extracts of the plant H.bacciferum has a antimicrobial effect against strains are listed And among the extracts, aqueous part is that most antibacterial effect of the other fraction and then methanolic extract has the greatest effect.

Key words: Heliotropium bacciferum, Antimicrobial activity, MIC, well plate.

Medicinal plants as scientific innovation, particularly in the medical field have found a special place. Currently, medicine and medical technology, has become highly dependent on each other. Although the diverse world of herbal medicine is not known as it deserves, but few studies have been done shows Critical value of the plant as a base for pharmaceutical science[1]

Iranian traditional medicine usually recommend a combination of herbal extracts, and often the combination of each individual patient, and he is determined according to the circumstances. Therapy with medicinal plants

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in Iran has a long history, so that the old Iranian medical sources such as the writings of EbneSina section is devoted to this topic[2]

Heliotropium bacciferum is One of the plants belonging to the family Boraginaceae, which is Restricted distribution in the south of Iran[3]. The antibacterial effect observed in other species of the genus *Heliotropium*. the antimicrobial activity of whole extracts and fractions (chloroform, Petroleum ether, ethyl acetate, methanol and aqueous) *H.bacciferum* against 5 bacterial strains such as *Staphylococcus aureus* (ATCC 6538), *Bacillus cereus* (ATCC 12826), *Escherichia coli* (ATCC 8739), *Salmonella enteritidis* (ATCC 13311) and *Pseudomonas aeruginosa* (ATCC 9027) using well plates and MIC in microplate to consider.

Alkaloid Heliotrine, transient hypotension in dogs and considerable reduction of nicotine causes a vasopressor response[4]. H.bacciferum is a rich source of pyrrolizidine alkaloids, some of which have anti-tumor, antimicrobial, anti-diabetic and antihyperlipedemic properties[5]

METHOD AND MATERIALS

After collecting plant and scientific identification, its aerial sections were dried, powdered. 50 mg of leaves removed And placed in 250 cc perculator, mixture with about 150 cc of 80% methanol. After 4 days extract obtained evacuated. Twice, each time with 150 ml of methanol Rinse the plant. It was 25 gr.

The extract obtained was concentrated at room temperature. The total extract extraction with petroleum ether, ethyl acetate and chloroform and aqueous. Extracts and fractions obtained were concentrated and finally dried. to study the antibacterial effect, stored in a clean, dark container and cool place and antibacterial effect were evaluated by determining the MIC and well plates.

For antimicrobial tests Was used of frozen Storage microbial cultures. Bacteria cultured on plates containing TSB medium, and incubated for 24 h at 37 °C. Microorganisms grow and become active. Then the culture plates containing fresh (one day) microorganisms used for antimicrobial testing.

Anti-microbial Evaluation cap plate Method

At this stage in the presence of a flame, by sterilized loop, clones were removed from the cultures of freshly prepared. Suspension was prepared in sterile normal saline.

So that the resulting turbidity equivalent to a standard 0.5 McFarland (0.5 mL of barium chloride 1.175 % + 99.5 mL of 1% sulfuric acid). The microbial suspension containing 1.5 x 10^8 Cfu/m bacteria. After Preparation Bacterial suspensions of each microbe, it poured in the plate to cool. Then on cultured each plate, 7 wells with sterile Pasteur pipette was created.

Samples obtained from plant extracts were prepared by the solvent DMSO 10%. Then builds into a well on plates containing medium by strile sampler, in first well 100 Landa and The second well 50 Landa, and to end well the 1.5 Landa was poured. for the filling of unfilled full well, add normal salin. The solvent DMSO 10% was used as a negative control. Gentamicin and Cephalexin was used as a positive control. After filling their plates, Required information written on them And put them in the oven 37° c to 24 hours.

Determine the MIC (Minimum inhibitory Concentration) using a microplate

4 mg of each extract poured in a small vial and mix with 2 ml DMSO 10%. Concentration is 2000 PPM. The solution used for the determination of MIC in microplate dilution method. In all well Poured 50 µl of DMSO 10% then added 50 µl of the extract in first well. Thus the concentration was 1000 µg / ml. 50 µl was removed from first well, added to the second wells and 50 µl removed to the third and so on.

Finally, 50µl of microbial suspension prepared in TSB added to each of the first row (Equivalent to 0.5 McFarland). In the next row, were Repeat for the other strains. As evidence used of Gentamicin, Cephalexin antibiotic. each dilution 100 µl Placed in separate houses microplate. The same procedure was repeated for the other extracts. All well contained 100 µl solution.

The task do for Rows 2 - 5 . Finally, microplate placed in zipper bag and put in the 37° c oven .24 hours later see the results. Notes the Wells where turbidity. As the MIC was reported clearly left last concentration Well.

RESULTS

Results of the MIC *H.bacciferum* with microplate method

1-1-Anti microbial effect of total extract

The results of antimicrobial effect of total extract showed that inhibits the growth of *Staphylococcus aureus* , *Bacillus cereus* and *Pseudomonas aeruginosa* in concentration of 15.625 µg per ml and *Escherichia coli* , *Salmonella enteritidis* in concentration of 31.25 µg per ml (Fig. 1 and Diagram 1).

Anti microbial effect of Chloroform extract

The results of antimicrobial effect of Chloroform extract showed that inhibits the growth of *Staphylococcus aureus*, *Bacillus cereus* and *Pseudomonas aeruginosa* in concentration of 15.625 µg per ml and *Salmonella enteritidis* in concentration of 62.5 µg per ml and *Escherichia coli* in concentration of 125 µg per ml (Fig. 2 and Diagram 1).

Anti microbial effect of Petroleum ether extract

The results of antimicrobial effect of Petroleum ether extract showed that inhibits the growth of *Staphylococcus aureus* , *Bacillus cereus* and *Pseudomonas aeruginosa* in concentration of 15.625 µg per ml and *Escherichia coli* in concentration of 125 µg per ml and *Salmonella enteritidis* in concentration of 62.5 µg per ml (Fig. 3 and Diagram 1).

Anti microbial effect of aqueous extract

The results of antimicrobial effect of

Table 1. MIC mean and standard deviation of total extract, chloroform, petroleum ether, aqueous and methanol plants Heliotropium bacciferum in serial concentrations of 1,000 to 0.035175 ug per ml and Gentamicin microdilution method in gram-negative bacteria *Escherichia coli*

t test	Genatmicin	Extract					Bacteria
p value		Metanolic	Aqueous	Petroleum ether	Chloroform	Total	gram negative
0.0001 < 0.0001	5.208±1.302				125±0.0	31.25±0.0	*E. coli*
0.0001 < 0.0438			5.208±20/83	125±0.0			
0.1481		2.6±10.42					

Lack of Growth concentration micrograms per milliliter (Mean ± standard deviation)

Table 2. MIC mean and standard deviation of total extract, chloroform, petroleum ether, aqueous and methanol plants Heliotropium bacciferum in serial concentrations of 1,000 to 0.035175 ug per ml and gentamicin microdilution method in gram-negative bacteria *Salmonella enteritidis*

t test	Genatmicin	Extract					Bacteria
p value		Metanolic	Aqueous	Petroleum ether	Chloroform	Total	gram negative
0.0001 < 0.0001	5.208±1.302				10.42±52.08	31.25±0.0	*Salmonella enteritidis*
0.0001 < 0.1161			7.813±0.0	0.0±6.25			
0.06		2.6±13.02					

Lack of Growth concentration micrograms per milliliter (Mean ± standard deviation)

aqueous extract showed that inhibits the growth of *Staphylococcus aureus* , *Bacillus cereus* and *Salmonella enteritidis* in concentration of 7.8125 µg per ml and *Escherichia coli, Pseudomonas aeruginosa* in concentration of 15.625 µg per ml (Fig. 4 and Diagram 1).

Table 3. MIC mean and standard deviation of total extract, chloroform, petroleum ether, aqueous and methanol plants Heliotropium bacciferum in serial concentrations of 1,000 to 0.035175 ug per ml and gentamicin microdilution method in gram-negative bacteria *Pseudomonas aeruginosa*

t test	Genatmicin	Extract					Bacteria
p value		Metanolic	Aqueous	Petroleum ether	Chloroform	Total	gram negative
0.0437						5.208± 20.83	*Pseudomonas aeruginosa*
0.0013	5/208±1/302				0.0±15.63		
0.0013				0.0±15.63			
0.06			2.604±13.02				
0.1161		0.0±7.813					

Lack of Growth concentration micrograms per milliliter (Mean ± standard deviation)

Table 4. MIC mean and standard deviation of total extract, chloroform, petroleum ether, aqueous and methanol plants Heliotropium bacciferum in serial concentrations of 1,000 to 0.035175 ug per ml and cefalexin microdilution method in gram- posetive bacteria *Staphylococcus aureus*

t test	Cefalexin	Extract					Bacteria
p value		Metanolic	Aqueous	Petroleum ether	Chloroform	Total	gram negative
0.06						5.208± 20.83	*S.aureus*
0.06	5.208±10.42				5.208±20.83		
0.0249				0.0±15.63			
0.0178			2.604±10.42				
0.0132		0.0±7.813					

Lack of Growth concentration micrograms per milliliter (Mean ± standard deviation)

Table 5. MIC mean and standard deviation of total extract, chloroform, petroleum ether, aqueous and methanol plants Heliotropium bacciferum in serial concentrations of 1,000 to 0.035175 ug per ml and gentamicin microdilution method in gram- posetive bacteria *Bacillus cereus*

t test	Cefalexin	Extract					Bacteria
p value		Metanolic	Aqueous	Petroleum ether	Chloroform	Total	gram negative
0.0890						5.208± 26.04	*S.aureus*
0.06	5.208±10.42				5.208±20.83		
0.0220				2.604±13.02			
0.0220			2.604±13.02				
0.0132		20.83±83.33					

Lack of Growth concentration micrograms per milliliter (Mean ± standard deviation)

Antimicrobial effect of metanolic extract

The results of antimicrobial effect of metanolic extract showed that inhibits the growth of *Staphylococcus aureus* , *Pseudomonas aeruginosa* in concentration of 7.8125 µg per ml and *Escherichia coli, Salmonella enteritidis* in concentration of 15.625 µg per ml and *Bacillus cereus* in concentration of 62.5 µg per ml (Fig. 5 and Diagram 1).

Results of the well plate *H.bacciferum* in gram-negative bacteria

Escherichia coli

The results in Table 1 indicate that the total extract at concentrations 31.25±0.0 micrograms per milliliter (ttest p value>0.0001), chloroform, petroleum ether fraction at concentrations 125±0.0 micrograms per milliliter(ttest p value>0.0001), aqueous fraction at concentrations 20.83±5.208 micrograms per milliliter (ttest p value>0.0001), metanolic fraction at concentrations 13.02±2.6 micrograms per milliliter(ttest p value=0.06) are prevent the growth of gram-negative bacteria *Salmonella enteritidis* (Table 2, diagram3).

Table 2. MIC mean and standard deviation of total extract, chloroform, petroleum ether, aqueous and methanol plants Heliotropium bacciferum in serial concentrations of 1,000 to 0.035175 ug per ml and gentamicin microdilution method in gram-negative bacteria *Salmonella enteritidis.*

Pseudomonas aeruginosa

The results in Table 3 indicate that the total extract at concentrations 20.83±5.208 micrograms per milliliter(ttest p value>0.0437), chloroform, petroleum ether fraction at concentrations 15.63±0.0 micrograms per milliliter (ttest p value>0.0013), aqueous fraction at concentrations 13.02±2.604 micrograms per milliliter (ttest p value>0.06), micrograms per milliliter(ttest p value=0.0438), methanol fraction at concentrations 10.42±2.6 micrograms per milliliter (ttest p value>0.1481) are prevent the growth of gram-negative bacteria *E. coli* (Table 1, Diagram2).

Table 1. MIC mean and standard deviation of total extract, chloroform, petroleum ether, aqueous and methanol plants Heliotropium bacciferum in serial concentrations of 1,000 to 0.035175 ug per ml and Gentamicin microdilution method in gram-negative bacteria *Escherichia coli*.

Salmonella enteritidis

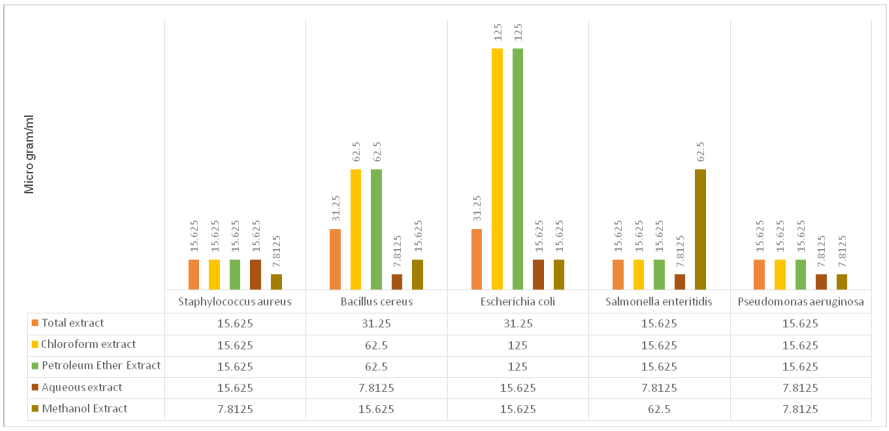
The results in Table 2 indicate that the total extract at concentrations 31.25±0.0 micrograms per milliliter (ttest p value>0.0001), chloroform fraction at concentrations 52.08±10.42 micrograms per milliliter (ttest p value>0.0111), petroleum ether fraction at concentrations 62.5±0.0

Table 6. The result of MIC H.bacciferum with microplate method

g/ml (µMIC	*Staphylococcus aureus*	*Bacillus cereus*	*Escherichia coli*	*Salmonella enteritidis*	*Pseudomonas aeruginosa*
total extract	15.75	15.75	31.25	31.25	15.75
Chloroform extract	15.75	15.75	125	62.5	15.75
petroleum ether extract	15.75	15.75	125	62.5	15.75
aqueous Extract	7.6	7.6	15.75	7.6	15.75
Methanol Extract	7.6	62.5	15.75	15.75	7.6

Table 6. The result of *H.bacciferum* well plate

g/ml (µMIC	*Staphylococcus aureus*	*Bacillus cereus*	*Escherichia coli*	*Salmonella enteritidis*	*Pseudomonas aeruginosa*
Total extract	31.25	31.25	62.5	62.5	31.25
Chloroform extract	31.25	7.6	62.5	62.5	31.25
petroleum ether extract	31.25	31.25	125	250	31.25
aqueous Extract	15.75	7.6	15.75	31.25	31.25
Methanol Extract	15.75	125	31.25	31.25	15.75



	Staphylococcus aureus	Bacillus cereus	Escherichia coli	Salmonella enteritidis	Pseudomonas aeruginosa
Total extract	15.625	31.25	31.25	15.625	15.625
Chloroform extract	15.625	62.5	125	15.625	15.625
Petroleum Ether Extract	15.625	62.5	125	15.625	15.625
Aqueous extract	15.625	7.8125	15.625	7.8125	7.8125
Methanol Extract	7.8125	15.625	15.625	62.5	7.8125

Diagram 1. MIC results of total extract, chloroform, petroleum ether, aqueous and methanol plants of Heliotropium bacciferum on five bacteria

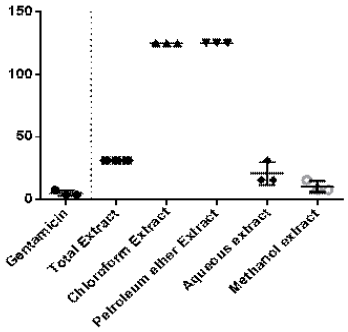


Diagram 2. MIC mean and standard deviation of total extract, chloroform, petroleum ether, aqueous and methanol plants Heliotropium bacciferum in serial concentrations of 1,000 to 0.035175 ug per ml and gentamicin microdilution method in gram-negative bacteria *Escherichia coli*

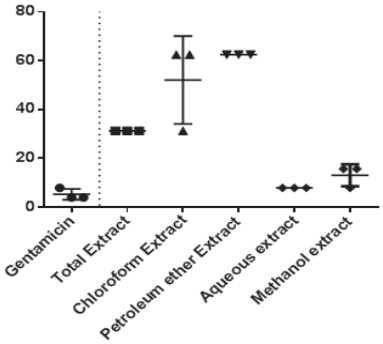


Diagram 3. MIC mean and standard deviation of total extract, chloroform, petroleum ether, aqueous and methanol plants Heliotropium bacciferum in serial concentrations of 1,000 to 0.035175 ug per ml and gentamicin microdilution method in gram-negative bacteria *Salmonella enteritidis*

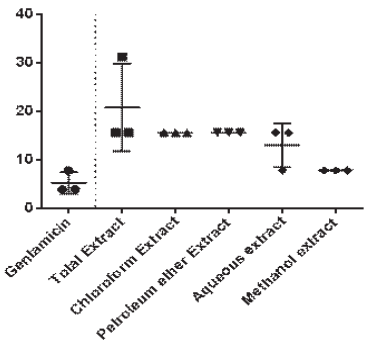


Diagram 4. MIC mean and standard deviation of total extract, chloroform, petroleum ether, aqueous and methanol plants Heliotropium bacciferum in serial concentrations of 1,000 to 0.035175 ug per ml and gentamicin microdilution method in gram-negative bacteria *Pseudomonas aeruginosa*

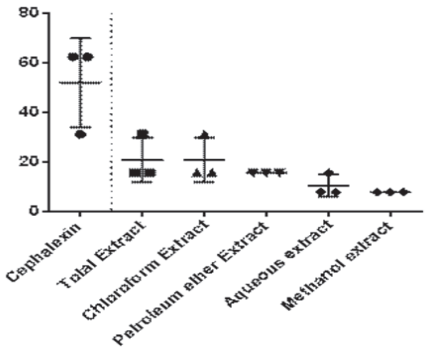


Diagram 5. MIC mean and standard deviation of total extract, chloroform, petroleum ether, aqueous and methanol plants Heliotropium bacciferum in serial concentrations of 1,000 to 0.035175 ug per ml and gentamicin microdilution method in gram-posetive bacteria *Staphylococcus aureus*

metanolic fraction at concentrations 7.813±0.0 micrograms per milliliter (ttest p value=0.1161) are prevent the growth of gram-negative bacteria *Pseudomonas aeruginosa* (table 3,diaagram4).

Table 3. MIC mean and standard deviation of total extract, chloroform, petroleum ether, aqueous and methanol plants Heliotropium bacciferum in serial concentrations of 1,000 to 0.035175 ug per ml and gentamicin microdilution method in gram-negative bacteria *Pseudomonas aeruginosa.*

Gram positive bacteria

Staphylococcus aureus

The results in Table 4 indicate that the petroleum ether at concentrations 15.63±0.0 micrograms per milliliter (ttest p value>0.0249),

0.035	0.061	0.122	0.244	0.488	0.976	1.953	3.906	7.812	15.62	31.25	62.5	125	250	500	1000	
Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Growth	Growth	Growth	Growth	Growth	Growth	Growth	Staphylococcus aureus
Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Growth	Growth	Growth	Growth	Growth	Growth	Escherichia coli
Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Growth	Growth	Growth	Growth	Growth	Salmonella enteritidis
Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Growth	Growth	Growth	Growth	Growth	Growth	Growth	Pseudomonas aeruginosa
Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Growth	Growth	Growth	Growth	Growth	Growth	Growth	Bacillus cereus

Fig. 1. Antimicrobial effect of total extract of the plant Heliotropium bacciferum in serial concentrations 1,000 to 0.035175 micrograms per milliliter

0.035	0.061	0.122	0.244	0.488	0.976	1.953	3.906	7.812	15.62	31.25	62.5	125	250	500	1000	
Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Growth	Growth	Growth	Growth	Growth	Growth	Growth	Staphylococcus aureus
Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Growth	Growth	Growth	Growth	Escherichia coli
Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Growth	Growth	Growth	Growth	Growth	Salmonella enteritidis
Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Growth	Growth	Growth	Growth	Growth	Growth	Growth	Pseudomonas aeruginosa
Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Growth	Growth	Growth	Growth	Growth	Growth	Growth	Bacillus cereus

Fig. 2. Antimicrobial effect of Chloroform extract of the plant Heliotropium bacciferum in serial concentrations 1,000 to 0.035175 micrograms per milliliter

0.035	0.061	0.122	0.244	0.488	0.976	1.953	3.906	7.812	15.62	31.25	62.5	125	250	500	1000	
Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Growth	Growth	Growth	Growth	Growth	Growth	Growth	*Staphylococcus aureus*
Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Growth	Growth	Growth	Growth	*Escherichia coli*
Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Growth	Growth	Growth	Growth	Growth	*Salmonella enteritidis*
Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Growth	Growth	Growth	Growth	Growth	Growth	Growth	*Pseudomonas aeruginosa*
Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Growth	Growth	Growth	Growth	Growth	Growth	Growth	*Bacillus cereus*

Fig. 3. Antimicrobial effect of Petroleum ether extract of the plant Heliotropium bacciferum in serial concentrations 1,000 to 0.035175 micrograms per milliliter

0.035	0.061	0.122	0.244	0.488	0.976	1.953	3.906	7.812	15.62	31.25	62.5	125	250	500	1000	
Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Growth	Growth	Growth	Growth	Growth	Growth	Growth	Growth	*Staphylococcus aureus*
Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Growth	Growth	Growth	Growth	Growth	Growth	Growth	*Escherichia coli*
Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Growth	Growth	Growth	Growth	Growth	Growth	Growth	Growth	*Salmonella enteritidis*
Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Growth	Growth	Growth	Growth	Growth	Growth	*Pseudomonas aeruginosa*
Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Growth	Growth	Growth	Growth	Growth	Growth	Growth	Growth	*Bacillus cereus*

Fig. 4. Antimicrobial effect of aqueous extract of the plant Heliotropium bacciferum in serial concentrations 1,000 to 0.035175 micrograms per milliliter

0.035	0.061	0.122	0.244	0.488	0.976	1.953	3.906	7.812	15.62	31.25	62.5	125	250	500	1000	
Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Growth	Growth	Growth	Growth	Growth	Growth	Growth	Growth	*Staphylococcus aureus*
Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Growth	Growth	Growth	Growth	Growth	Growth	Growth	*Escherichia coli*
Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Growth	Growth	Growth	Growth	Growth	Growth	Growth	*Salmonella enteritidis*
Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Growth	Growth	Growth	Growth	Growth	Growth	Growth	Growth	*Pseudomonas aeruginosa*
Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Lack of Growth	Growth	Growth	Growth	Growth	Growth	*Bacillus cereus*

Fig. 5. Antimicrobial effect of metanolic extract of the plant Heliotropium bacciferum in serial concentrations 1,000 to 0.035175 micrograms per milliliter

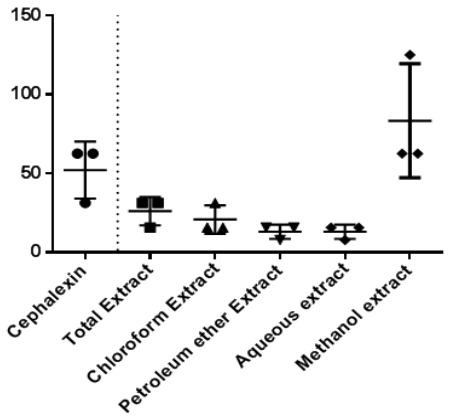


Diagram 6. MIC mean and standard deviation of total extract, chloroform, petroleum ether, aqueous and methanol plants Heliotropium bacciferum in serial concentrations of 1,000 to 0.035175 ug per ml and cefalexin microdilution method in gram-posetive bacteria *Bacillus cereus*

aqueous fraction at concentrations 10.42±2.604 micrograms per milliliter (ttest p value>0.0178), metanolic fraction at concentrations 7.813±0.0 micrograms per milliliter (ttest p value>0.0132),total extract, Chloroform fraction at concentrations 20.83±5.208 micrograms per milliliter(ttest p value=0.06) are prevent the growth of gram-posetive bacteria *Staphylococcus aureus* (table 4,diagram5).

Table 4. MIC mean and standard deviation of total extract, chloroform, petroleum ether, aqueous and methanol plants Heliotropium bacciferum in serial concentrations of 1,000 to 0.035175 ug per ml and cefalexin microdilution method in gram- posetive bacteria *Staphylococcus aureus.*

Bacillus cereus

The results in Table 5 indicate that the petroleum ether, aqueous at concentrations 13.02±2.604 micrograms per milliliter(ttest p value=0.0220), metanolic fraction at concentrations 83.33±20.83 micrograms per milliliter(ttest p value=0.0132), total extract at concentrations 26.04±5.208 micrograms per milliliter(ttest p value=0.0890), Chloroform fraction at concentrations 20.83±5.208 micrograms per milliliter(ttest p value=0.06) are prevent the growth of gram-posetive bacteria *Bacillus cereus* (table 5, diagram 6).

Table 5. MIC mean and standard deviation of total extract, chloroform, petroleum ether, aqueous and methanol plants Heliotropium bacciferum in serial concentrations of 1,000 to 0.035175 ug per ml and gentamicin microdilution method in gram- posetive bacteria *Bacillus cereus.*

DISCUSSION

The research was carried out on other species of the genus Heliotropium, this species has an antibacterial effect such as: H.indicum, the results are remarkable antimicrobial against Gram positive and Gram negative bacteria. The alkaloid compounds have anti-inflammatory, wound healing, antiseptic and antibacterial effect. Eicosapentenoic acid which is one omega-3 fatty acid in this plant, sterilizing wounds and protecting wounds against microbes[6]. Phytochimical analysis of all extracts was determined that the antimicrobial activity is due to the presence of phenolic compounds[7]. Antibacterial effect of plant extracts H.sinuatum has been associated with long-chain alcohol and ketones compounds[8]. The antimicrobial effect of H.ellipticum has been linked to petrol and triterpenoid in this plant[9].

In a study, the effects of chloroform, ethyl acetate, methanol and aqueous extract of Heliotropium marifolium were investigated. The results showed antimicrobial properties[10].

Four pyrrolizidine alkaloid isolated from *Heliotropium bacciferum* determind as Heleurine¡ Heliotrine¡ Supinine and Europine[11]. Given that past research has been done on the effects of various extracts and essential oils from various plants (12 to 18), and research on Anti-bacterial plant extracts has been determined and compared with other herbs, the results of this study indicate that extracts of the plant *H.bacciferum* has a antimicrobial effect against strains *Staphylococcus aureus* , *Bacillus cereus, Escherichia coli*, *Salmonella enteritidis* and *Pseudomonas aeruginosa,* And among the extracts, aqueous part is that most antibacterial effect of the other fraction and then methanolic extract has the greatest effect.

All microorganisms show MIC 62.5-7.6 µg/ml except fractions chloroform and petroleum ether on E. coli is 125 µg/ml.

According to studies on the plant, expected to have an antimicrobial effect. Therefore suggested isolation and purified antimicrobial

compound in the extract and identify the main cause of Anti-microbial agent. determine the molecular structure of these compounds to introduced Effective antimicrobial products. It is proposed to investigate the antimicrobial effect of other fractions of the plant.

It is hoped that in the future, done additional research on the antimicrobial activity of different species of the plant and by finding antimicrobial active ingredients of the plant and formulations, preparation the dosage form, to be taken an important step towards diseases that are caused by various species of bacteria.

CONCLUSION

The results of this study indicate that the antimicrobial effect of aqueous fraction of Heliotropium bacciferum Far more than any other plant extracts and then antimicrobial effect of methanol fraction is greater than the other.

According to studies done in the past on other plant species has been observed that the anti-bacterial effect of this plant on *Staphylococcus aureus, Bacillus cereus, Escherichia col, Salmonella enteritidis, Pseudomonas aeruginosa.*

REFERENCES

1. Zahdad B. Geno Protected Area. Publications Department of the Environment. 1375, P 70 .
2. Davies J, Webb V. The Emerging infection. Sandiego: Academic press; 1998. chapter 8, p 72-229.
3. Mozaffarian V. Culture of Iran's scientific names of plants. Fifth Edition. Print Ghadiani; 1386, P.211.
4. Zargari A. Herb. Seventh Edition. Issued of Tehran University; 1390, pp. 549-526.
5. Khasawneh M, Hamza A, Fawzi N. Antioxidant activity and phenolic content of some emirates medicinal plants. *Advances in Food Sciences*. 2010. **32** :62-66.
6. Oluwatoyin SM, leogbulam NG, Joseph A. Phytochemical and antimicrobial studies on the aerial part of *Heliotropium indicum Linn.Annal. biol.res.* 2001; **2**(2) :129-136
7. Nethaji S, Manokaran C.Phytochemical analysis and antimocrobial activity of *Heliotropium indicum L.* and *Coldenia procumbens L. Journal of Pure and Applied Microbiology.* 2009; **3**(1): 195-198.
8. Modak B, Torres R, Wilkens M, Urzua A. Antimicrobial activity of compounds isolated of the resinous exudate from *Heliotropium sinuatum* on phytopathogenic bacterial. *J. Chil. Chem. Soc.* 2003; **49**(1) :717-720.
9. Jain Sc, Singh B, Jain R. Antimicrobial activity of tritopenoids from *Heliotropium ellipticum*. *Fitotrapia*.2001; **72**(6): 666-668.
10. Radha1 R, Lata2 T, Rajendran N. N. Antimicrobial Activity of Crude Extracts of *Heliotropium marifolium* Retz. *Journal of natural remedies.* 2003; **3**(2): 208-211.
11. Farrag N M, Abdel-Aziz E M, El-Shafae A M, Ateya A M and Domiaty M M El.Pyrrolizidine Alkaloids of *Heliotropium bacciferum Forssk* from Egypt, 1996; **34**(5): 374-377.
12. Larypoor M, Akhavansepahy A, Rahimifard N, Rashedi H. Antidermatophyte activity of the essential oil of *Hypericum perforatum* of North of Iran. *Journal of Medicinal plants.* 2009; **8**(31): 110-117
13. Rahimifard N, Sabzevari O, Shoeibi Sh, Pakzad SR, Ajdari, S, Hajimehdipoor, H, Bagheri F. and Safaee M. Antifungal activity of the essential oil of *Eugenia caryophyllata* on *Candida albicans*, *Aspergillus niger* and *Aspergillus flavus*. *Biomed Pharmacol. J.* 2008; **1**(1): 43-46.
14. Rahimifard N, Sabzevari O, Shoeibi Sh, Pakzad SR, Ajdari S, Hajimehdipoor, H, Bagheri F and Bagheri A. Antifungal activity of the essential oil of *Cinnamomum zeylanicum* on *Candida albicans*, *Aspergillus niger* and *Aspergillus flavus*. *Biomedical and Pharmacology Journal.* 2008; **1**(1): 85-88.
15. Rahimifard, N. Antifungal activity of native essential oil of *Zataria multiflora*on *Candida albicans, Aspergillus niger* and *Aspergillus flavus* from Iran. *Biomedical and Pharmacology Journal.;* 2008: **1**(02): 289-292.
16. Rahimifard, N. Antifungal activity of native essential oil of *Thymus vulgaris* on *Candida albicans, Aspergillus niger* and *Aspergillus flavus* from Iran. *Journal of pure and applied microbiology.* 2008; **2**(02): 343-346.
17. Shoeibi, Sh., Hajimehdipoor, H., Rahimifard, N., Rezazadeh, S.H., Hasanloo, T., Bagheri, F., Amini, A.Comparative study on anti-Helicobacter pylori effects of licorice roots collected from different regions of Iran, *Journal of Medicinal Plants ,* 2010; **9**(36): 43-47+214 .
18. Rahimifard, N., Rabiei, M., Beitolahi, L., Ahi, K.*Helicobacter pylori* and the herbal compound effect., *Biosciences Biotechnology Research Asia,* 2012; **7**(02): 647-649.